

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
3 January 2002 (03.01.2002)

PCT

(10) International Publication Number
WO 02/00690 A2

(51) International Patent Classification ⁷ :	C07K 14/00	09/854,208	10 May 2001 (10.05.2001)	US
(21) International Application Number:	PCT/US01/19692	09/854,280	10 May 2001 (10.05.2001)	US
(22) International Filing Date:	20 June 2001 (20.06.2001)	09/866,028	25 May 2001 (25.05.2001)	US
(25) Filing Language:	English	09/866,034	25 May 2001 (25.05.2001)	US
(26) Publication Language:	English	PCT/US01/17092	25 May 2001 (25.05.2001)	US
(30) Priority Data:		09/870,574	30 May 2001 (30.05.2001)	US
60/213,637	23 June 2000 (23.06.2000)	US	PCT/US01/17443	30 May 2001 (30.05.2001)
60/219,556	20 July 2000 (20.07.2000)	US	PCT/US01/17800	1 June 2001 (01.06.2001)
60/220,624	25 July 2000 (25.07.2000)	US		US
60/220,664	25 July 2000 (25.07.2000)	US		
PCT/US00/20710	28 July 2000 (28.07.2000)	US		
60/222,695	2 August 2000 (02.08.2000)	US		
09/643,657	17 August 2000 (17.08.2000)	US		
PCT/US00/23522	23 August 2000 (23.08.2000)	US		
PCT/US00/23328	24 August 2000 (24.08.2000)	US		
60/230,978	7 September 2000 (07.09.2000)	US		
60/000,000	15 September 2000 (15.09.2000)	US		
09/664,610	18 September 2000 (18.09.2000)	US		
09/665,350	18 September 2000 (18.09.2000)	US		
60/242,922	24 October 2000 (24.10.2000)	US		
09/709,238	8 November 2000 (08.11.2000)	US		
PCT/US00/30952	8 November 2000 (08.11.2000)	US		
PCT/US00/30873	10 November 2000 (10.11.2000)	US		
PCT/US00/32678	1 December 2000 (01.12.2000)	US		
09/747,259	20 December 2000 (20.12.2000)	US		
PCT/US00/34956	20 December 2000 (20.12.2000)	US		
09/767,609	22 January 2001 (22.01.2001)	US		
09/796,498	28 February 2001 (28.02.2001)	US		
PCT/US01/06520	28 February 2001 (28.02.2001)	US		
PCT/US01/06666	1 March 2001 (01.03.2001)	US		
09/802,706	9 March 2001 (09.03.2001)	US		
09/808,689	14 March 2001 (14.03.2001)	US		
09/816,744	22 March 2001 (22.03.2001)	US		
09/828,366	5 April 2001 (05.04.2001)	US		

(71) Applicant (for all designated States except US): GENEN-TECH, INC. [US/US]; 1 DNA Way, South San Francisco, CA 94080-4990 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): BAKER, Kevin, P. [GB/US]; 14006 Indian Run Drive, Darnestown, MD 20878 (US). FERRARA, Napoleone [US/US]; #704, 2090 Pacific Avenue, San Francisco, CA 94109 (US). GERBER, Hanspeter [CH/US]; #5, 1121 Tennessee Street, San Francisco, CA 94107 (US). GERRITSEN, Mary, E. [CA/US]; 541 Parrott Drive, San Mateo, CA 94402 (US). GODDARD, Audrey [CA/US]; 110 Congo Street, San Francisco, CA 94131 (US). GODOWSKI, Paul, J. [US/US]; 2627 Easton Drive, Burlingame, CA 94010 (US). GURNEY, Austin, L. [US/US]; 1 Debbie Lane, Belmont, CA 94002 (US). HILLAN, Kenneth, J. [GB/US]; 64 Seward Street, San Francisco, CA 94114 (US). MARSTERS, Scot, A. [US/US]; 990 Cherry Street, San Carlos, CA 94070 (US). PAN, James [CA/US]; 2705 Coronet Boulevard, Belmont, CA 94002 (US). PAONI, Nicholas, F. [US/US]; 1756 Terrace Drive, Belmont, CA 94002 (US). STEPHAN, Jean-Philippe, F. [FR/US]; 320 C Lansdale Avenue, Millbrae, CA 94030 (US). WATAN-ABE, Colin, K. [US/US]; 128 Corliss Drive, Moraga, CA 94556 (US). WILLIAMS, P., Mickey [US/US]; 509 Alto Avenue, Half Moon Bay, CA 94019 (US). WOOD, William, I. [US/US]; 35 Southdown Court, Hillsborough, CA 94010 (US). YE, Weilan [CN/US]; 119 Barkentine Street, Foster City, CA 94404 (US).

(74) Agents: AGARWAL, Atulya, R. et al.; c/o GENEN-TECH, INC., MS49, 1 DNA Way, South San Francisco, CA 94080-4990 (US).

[Continued on next page]

WO 02/00690 A2

(54) Title: COMPOSITIONS AND METHODS FOR THE DIAGNOSIS AND TREATMENT OF DISORDERS INVOLVING ANGIOGENESIS

(57) Abstract: Compositions and methods are disclosed for stimulating or inhibiting angiogenesis and/or cardiovascularization in mammals, including humans. Pharmaceutical compositions are based on polypeptides or antagonists thereto that have been identified for one or more of these uses. Disorders that can be diagnosed, prevented, or treated by the compositions herein include trauma such as wounds, various cancers, and disorders of the vessels including atherosclerosis and cardiac hypertrophy. In addition, the present invention is directed to novel polypeptides and to nucleic acid molecules encoding those polypeptides. Also provided herein are vectors and host cells comprising those nucleic acid sequences, chimeric polypeptide molecules comprising the polypeptides of the present invention fused to heterologous polypeptide sequences, antibodies which bind to the polypeptides of the present invention and to methods for producing the polypeptides of the present invention.



(81) **Designated States (national):** AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report*

(84) **Designated States (regional):** ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

6.20. EXAMPLE 20: Normal Human Iliac Artery Endothelial Cell Proliferation (Assay 138)

This assay is designed to determine whether PRO polypeptides of the present invention show the ability to induce proliferation of human iliac artery endothelial cells in culture and, therefore, function as useful growth factors.

5 On day 0, human iliac artery endothelial cells (from cell lines, maximum of 12-14 passages) were plated in 96-well plates at 1000 cells/well per 100 microliter and incubated overnight in complete media [epithelial cell growth media (EGM, Clonetics), plus supplements: human epithelial growth factor (hEGF), bovine brain extract (BBE), hydrocortisone, GA-1000, and fetal bovine serum (FBS, Clonetics)]. On day 1, complete media was replaced by basal media [EGM plus 1% FBS] and addition of PRO polypeptides at 1%, 0.1% and 0.01%. On 10 day 7, an assessment of cell proliferation was performed by Alamar Blue assay followed by Crystal Violet. Results are expresses as % of the cell growth observed with control buffer.

The following PRO polypeptides tested positive in this assay: PRO214, PRO238, PRO256, PRO363, PRO365, PRO791, PRO836, PRO1025, PRO1029, PRO1186, PRO1192, PRO1272, PRO1274, PRO1279, PRO1306, PRO1325, PRO1329, PRO1376, PRO1411, PRO1419, PRO1508, PRO1787, PRO1868, PRO1890, 15 PRO4324, PRO4333, PRO4408, PRO4499, PRO5725, PRO6006, PRO9821, PRO9873, PRO10008, PRO10096, PRO19670, PRO20040, PRO20044, PRO21384 and PRO28631.

6.21. EXAMPLE 21: Pooled Human Umbilical Vein Endothelial Cell Proliferation (Assay 139)

This assay is designed to determine whether PRO polypeptides of the present invention show the ability to induce proliferation of pooled human umbilical vein endothelial cells in culture and, therefore, function as useful growth factors.

20 On day 0, pooled human umbilical vein endothelial cells (from cell lines, maximum of 12-14 passages) were plated in 96-well plates at 1000 cells/well per 100 microliter and incubated overnight in complete media [epithelial cell growth media (EGM, Clonetics), plus supplements: human epithelial growth factor (hEGF), bovine 25 brain extract (BBE), hydrocortisone, GA-1000, and fetal bovine serum (FBS, Clonetics)]. On day 1, complete media was replaced by basal media [EGM plus 1% FBS] and addition of PRO polypeptides at 1%, 0.1% and 0.01%. On day 7, an assessment of cell proliferation was performed by Alamar Blue assay followed by Crystal Violet. Results are expresses as % of the cell growth observed with control buffer.

The following PRO polypeptides tested positive in this assay: PRO181, PRO205, PRO221, PRO229, 30 PRO231, PRO238, PRO241, PRO247, PRO256, PRO258, PRO263, PRO265, PRO295, PRO321, PRO322, PRO337, PRO363, PRO444, PRO533, PRO697, PRO725, PRO771, PRO788, PRO819, PRO827, PRO828, PRO846, PRO865, PRO1005, PRO1006, PRO1007, PRO1025, PRO1054, PRO1071, PRO1075, PRO1079, PRO1080, PRO1114, PRO1131, PRO1155, PRO1160, PRO1184, PRO1190, PRO1192, PRO1195, PRO1244, PRO1272, PRO1273, PRO1279, PRO1283, PRO1286, PRO1306, PRO1309, PRO1325, PRO1329, PRO1347, 35 PRO1356, PRO1376, PRO1382, PRO1412, PRO1419, PRO1474, PRO1477, PRO1488, PRO1550, PRO1556, PRO1760, PRO1782, PRO1787, PRO1801, PRO1868, PRO1887, PRO3438, PRO3444, PRO4302, PRO4324, PRO4341, PRO4342, PRO4353, PRO4354, PRO4356, PRO4371, PRO4405, PRO4422, PRO4425, PRO5723,

PRO5725, PRO5737, PRO5776, PRO6029, PRO6071, PRO7436, PRO9771, PRO10008, PRO10096, PRO21055 and PRO21384.

6.22. EXAMPLE 22: Human Coronary Artery Smooth Muscle Cell Proliferation (Assay 140)

This assay is designed to determine whether PRO polypeptides of the present invention show the ability to induce proliferation of human coronary artery smooth muscle cells in culture and, therefore, function as useful growth factors.

On day 0, human coronary artery smooth muscle cells (from cell lines, maximum of 12-14 passages) were plated in 96-well plates at 1000 cells/well per 100 microliter and incubated overnight in complete media [smooth muscle growth media (SmGM, Clonetics), plus supplements: insulin, human epithelial growth factor (hEGF), human fibroblast growth factor (hFGF), GA-1000, and fetal bovine serum (FBS, Clonetics)]. On day 1, complete media was replaced by basal media [SmGM plus 1% FBS] and addition of PRO polypeptides at 1%, 0.1% and 0.01%. On day 7, an assessment of cell proliferation was performed by Alamar Blue assay followed by Crystal Violet. Results are expresses as % of the cell growth observed with control buffer.

The following PRO polypeptides tested positive in this assay: PRO162, PRO181, PRO182, PRO195, PRO204, PRO221, PRO230, PRO256, PRO258, PRO533, PRO697, PRO725, PRO738, PRO826, PRO836, PRO840, PRO846, PRO865, PRO982, PRO1025, PRO1029, PRO1071, PRO1080, PRO1083, PRO1134, PRO1160, PRO1182, PRO1184, PRO1186, PRO1192, PRO1265, PRO1274, PRO1279, PRO1283, PRO1306, PRO1308, PRO1309, PRO1325, PRO1337, PRO1338, PRO1343, PRO1376, PRO1387, PRO1411, PRO1412, PRO1415, PRO1434, PRO1474, PRO1488, PRO1550, PRO1556, PRO1567, PRO1600, PRO1754, PRO1758, PRO1760, PRO1787, PRO1865, PRO1868, PRO1917, PRO1928, PRO3438, PRO3562, PRO4302, PRO4333, PRO4345, PRO4353, PRO4354, PRO4405, PRO4408, PRO4430, PRO4503, PRO5725, PRO6714, PRO9771, PRO9820, PRO9940, PRO10096, PRO21055, PRO21184 and PRO21366.

6.23. EXAMPLE 23: Microarray Analysis to Detect Overexpression of PRO Polypeptides in HUVEC Cells Treated with Growth Factors

This assay is designed to determine whether PRO polypeptides of the present invention show the ability to induce angiogenesis by stimulating endothelial cell tube formation in HUVEC cells.

Nucleic acid microarrays, often containing thousands of gene sequences, are useful for identifying differentially expressed genes in tissues exposed to various stimuli (e.g., growth factors) as compared to their normal, unexposed counterparts. Using nucleic acid microarrays, test and control mRNA samples from test and control tissue samples are reverse transcribed and labeled to generate cDNA probes. The cDNA probes are then hybridized to an array of nucleic acids immobilized on a solid support. The array is configured such that the sequence and position of each member of the array is known. Hybridization of a labeled probe with a particular array member indicates that the sample from which the probe was derived expresses that gene. If the hybridization signal of a probe from a test (exposed tissue) sample is greater than hybridization signal of a probe from a control (normal, unexposed tissue) sample, the gene or genes overexpressed in the exposed tissue are identified. The